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Variability in growth, nutrition and phytochemical constituents of *Plectranthus amboinicus* (Lour) Spreng. as influenced by indigenous arbuscular mycorrhizal fungi

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Abstract: A study was conducted under greenhouse nursery condition on the efficacy of seven indigenous arbuscular mycorrhizal (AM) fungi in the improvement of growth, biomass, nutrition and phytochemical constituents, namely total phenols, ortho dihydroxy phenols, flavonoids, alkaloids, tannins and saponins, in the roots and leaves of *Plectranthus amboinicus* (Lour) Spreng. Seedlings were raised in polythene bags containing soil inoculated with isolates of seven different indigenous AM fungi, viz. *Acaulospora bireticulata, A. scrobiculata, Gigaspora margarita, Glomus aggregatum, G. mosseae, G. geosporum*, *and Scutellospora heterogama*. *P. amboinicus* seedlings raised in the presence of AM fungi generally showed an increase in plant growth, nutritional status and phytochemical constituents over those grown in the absence of AM fungi. The extent of growth, biomass, nutritional status and phytochemical constituents enhanced by AM fungi varied with the species of AM fungi inhabiting the roots and leaves of *P. amboinicus* seedlings. Considering the various plant growth parameters, nutritional status of the plant, total phenols, ortho dihydroxy phenols, alkaloids , flavonoids , tannins, and saponins in the roots and leaves, it was observed that *Gigaspora margarita* is the best AM symbiont for *P. amboinicus* used in this experiment.

Keywords: *Plectranthus amboinicus*, AM fungi, biomass, nutrition, phytochemicals

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Introduction

The introduction of beneficial organisms into soil is a present crux of applied mycorrhizal research. Utilisation of mycorrhizal biofertilisers in the cultivation of medicinal and aromatic plants is of recent interest. Arbuscular mycorrhizal (AM) fungi have been used to enhance the plant growth and yield of medicinal crops and to help maintain good soil health and fertility that contributes to a greater extent to a sustainable yield and good quality of the products [1]. The activity has gained momentum in recent years due to the higher cost and hazardous effects of heavy doses of chemical fertilisers. These fungi are a ubiquitous group of soil fungi colonising the roots of plants belonging to more than 90% of the plant families [2]. These zygomycetous fungi represent an important component in the soil microbial biomass due to their ubiquity and their direct involvement in essential processes at the plant-soil interface [3]. Interest in this association is mainly because of the manifold benefits conferred on the host by the fungi. They are known to improve the nutritional status of plants as well as their growth and development, and to protect plants against root pathogens and confer resistance to drought and soil saline condition [4]. With over 130 species of AM fungi recognised and classified [5] and the wide host range they inhabit, there exists a wide variation in the ways they benefit the host, which in turn are related to the extent of the colonisation of host roots by the fungus. The extent of the root colonisation varies with several soil and climatic factors apart from the host involved. However, these fungi show a preferential colonisation to hosts and thus the extent to which the host benefits depends of the fungal species involved in the symbiosis [6]. The existence of inter- and intra-specific variations among the plant species involved in relation to their phosphorus requirement and the ability of the host to translocate the native soil phosphorus further determine the efficacy of these fungi [7]. Thus it is essential to screen for an efficient AM fungus for a particular host in order to harness the maximum benefit from the fungus. Furthermore, since AM fungi cannot be grown on laboratory media, production of a large quantity of the inoculum for inoculation of the soil under field conditions is difficult. Nevertheless, since most of the commercially important medicinal crops are raised under nursery conditions before being transplanted to the main field, the inoculation of soil in the nursery would not only result in the saving of the inoculum needed but also help in better establishment of the transplanted seedlings.

There are few published reports on the influence of AM fungi on the growth, nutrition and phytochemical constituents of medicinal plants [1,8,9,]. Indian borage (*Plectranthus amboinicus* Lour. Spreng.) is an important medicinal plant largely used in Indian siddha medicine and the leaves are used for the treatment of urinary diseases, epilepsy , chronic asthma, cough, bronchitis, and malarial fever, in addition to acting as a powerful aromatic carminative. Hence the present investigation was done to screen for an efficient AM fungus for *P. amboinicus* and also to study the effects of the association on the growth, biomass, nutritional status and phytochemicals, viz. total phenols, ortho di-hydroxy phenols, alkaloids, flavonoids, tannins and saponins in the leaves and roots of *P. amboinicus*.

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Materials and Methods

This investigation was carried out under nursery condition in a glass house. The soil used in this study was collected from an uncultivated field at a depth of 0-30 cm and was classified as fine, entisol, isohyperthermic kanhaplustalfs. The soil pH was 7.2 (1:10 soil to water extract ratio), and it contained 2.7 ppm available phosphorus (extractable with NH_4F + HCl) and an indigenous AM fungal population of 60 spores/50 g of soil. Nursery was raised by sterilising the seeds of *P. amboinicus* with 5% chloramine T solution for 30 min, then washing and sowing in poly bags (10 x 15 cm) containing sterilised soil: vermiculite mix (1:1v/v). Ruakura nutrient solution at 50ml per poly bag was applied once in 10 days. After 30 days seedlings were transplanted to polythene bags of size 25 x 15 cm containing 2 kg of unsterilised soil:sand:compost in the ratio of 2:1:0.5 (v/v/v).

The AM fungal species used in this study (Table 1) were isolated from the rhizosphere soil of Indian borage cultivated at the herbal garden of Tamil University, Tamilnadu, India. These AM fungal species were isolated by using wet sieving and decanting technique [10]. The species level identification of different AM fungal species was done following the keys provided by Trappe [11] and Schenck and Perez [12]. These fungi were multiplied using sterilised sand:soil mix (1:1 v/v) as the substrate and onion as the host. After 90 days of growth, shoots of onion was severed and the substrate containing hyphae, spores and root bits was air dried and used as inoculum. The inoculum potential (IP) of each culture was estimated adopting the Most Probable Number (MPN) method as outlined by Porter [13]. The soil in each polythene bag was mixed with this inoculum at different rates so as to maintain an initial IP of 12,500 per polythene bag. Each bag containing the potting mixture, with or without AM inoculum as the treatment may be, was planted with one seedling of *P. amboinicus*. One set of plants without inoculation was the control. Each treatment with 5 replications was maintained in a glass house and watered regularly so as to maintain the field capacity of the soil. Ruakura plant nutrient solution [3] without phosphate was added to the polythenebags at the rate of 50 ml per polythene bags once in 15 days.

Ninety days after transplanting, the plants were harvested for determination of the mycorrhizal status, growth response, nutritional status and phytochemical constituents. Plant height was measured from soil surface to the growing tip of the plant. Dry biomass was determined after drying the plant sample at 60ºC to a constant weight in a hot air oven. Soil sample (50 g) was collected from each polythene bag and subjected to wet sieving and decantation method as outlined by Gerdemann and Nicolson [10] to estimate the population of spores. The root system was removed and assessed for AM fungal infection by the grid-line intersect method [14] after clearing the roots with 10% KOH and staining with trypan blue (0.02%) as described by Phyllips and Hayman [15]. Estimation of soil aggregates (<50µm size), which indirectly denote the extent of external hyphae in soil, was done as described by Van Bavel [16].

Phosphorus and potassium content of the plant tissue were determined by employing the vanadomolybdate phosphoric yellow colour and flame photometric method [17] respectively. Atomic absorption spectrophotometry was employed to estimate zinc, copper and iron content of the plant

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samples, using respective hollow cathode lamps. Sturdiness quotient, biovolume index (a measure of the total volume of a seedling), and quality index, which reflects the quality of a seedling, were determined using the formulae given by Hatchell [18]. The content of secondary metabolites, viz. total phenols [19], ortho dihydroxy phenols [20], flavonoids, tannins, saponins, and alkaloids in the leaves of the tested plants, were assayed according to the methods described by Sadasivam and Manickam [21] and Zakaria [22]. The data thus generated were subjected to statistical analysis of completely randomised block design and the means were separated by Duncan's Multiple Range Test (DMRT) [23].

Results and Discussion

In the field survey, Indian borage plants growing in uncultivated P-deficient sandy loam soils were almost the same as those growing in cultivated soils. Microscopic examination of their roots revealed extensive colonisation by AM fungi with 94.5% level of infection. A large number of inter and intra-matrical vesicles were noticed between 120µm and 140µm in size. The vesicles were globose to subglobose and the subtending hyphae were simple. Based on the morphological characters, the AM fungal isolate was identified as *Glomus* species. Altogether seven AM fungi were isolated from root-zone soils and identified (Table 1). Among them *Glomus aggregatum* and *Gigaspora margarita* were predominant. However, *Acaulospora* and *Scutellospora* rarely occurred. Soil-borne auxiliary cells of *Gigaspora* and *Scutellospora* were also isolated and identified.

Table 1. Different native AM fungi and their influence on growth of *P. amboinicus*

Treatment	Plant height (cm)		Plant dry weight (g/plant)	
	Shoot	Root	Shoot	Root
Control (without AM fungi)	62.0a	23.5a	16.5a	12.5a
Acaulospora bireticulata	68.6b	28.6b	19.8b	18.2b
Acaulospora scrobiculata	66.4b	28.2b	18.8b	17.5c
Gigaspora margarita	90.6d	52.4d	24.5d	23.2d
Glomus aggregatum	84.5cd	42.0cd	23.8c	19.9b
Glomus geosporum	72.4c	34.5c	20.2bc	19.8b
Glomus mosseae	86.5d	46.2cd	24.0c	20.4cd
Scutellospora heterogama	68.5b	29.2b	19.2b	17.6c

Note: Means (n = 5) in each column followed by the same letter are not significantly different ($p < 0.05$) from each other according to DMR test.

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The growth response, nutritional status and mycorrhizal development of plants raised in sandy loam soils were assessed for the impact of inoculation with different native AM fungi. The responses of the Indian borage plants to inoculation with different AM fungi were found to be varied. Mycorrhizal inoculation resulted in a significant increase in height, biomass, nutrient content and phytochemical constituents of *P. amboinicus* seedlings. However, there was no positive correlation between plant growth parameters and mycorrhizal colonisation. Earlier studies also showed the same trend for medicinal plants subjected to AM inoculation [1,9, 24-25] and these studies also indicated the host preference to the AM fungi. Bagyaraj and Varma [4] and Jeffries [26] stressed the need for selecting efficient native AM fungi for plant species. The present study conducted with an objective of screening for an efficient indigenous AM fungi for *P. amboinicus* seedlings has also resulted in varied plant growth responses to different AM fungi.

Mycorrhizal treatments resulted in an increase in the number of spores in the rhizosphere soils and this was maximum with *Gigaspora margarita* followed by *Glomus mosseae*. It is well known that enhanced nutritional status of a plant is manifested in its improved growth [26]. *P. amboinicus* plants grown in the presence of AM fungi showed a general increase in such growth parameters as plant height and total dry weight as against those grown in soils uninoculated with AM fungi (Table 1).
The nutritional status of *P. amboinicus* seedlings, viz. phosphorus, potassium, zinc, copper and iron content, was also significantly higher in plants raised in soil inoculated with AM fungi (Table 2). Seedlings raised in the presence of *Gigaspora margarita* showed an increase of 108%, 81%, 82%, 80.5% and 82.5% in the tissue P, K, Zn, Cu and Fe content respectively as compared to seedlings raised as uninoculated control. The extent of increase in plant P, K, Zn, Cu and Fe content varied among the fungi studied with seedlings grown in the presence of *Gigaspora margarita* containing significantly highest content of these nutrients, followed by those grown in the presence of *Glomus aggregatum* and *G. mosseae*. Such a variation in the plant nutrient status in relation to the fungal species for other medicinal plant species is well documented [1,24]. The enhancement in growth and nutritional status is also related to the per cent root colonisation apart from several soil and environmental factors.

The phytochemical constituents total phenols (ortho di-hydroxy phenols, flavonoids, alkaloids, tannins and saponins) of *P. amboinicus* seedlings were found to be significantly higher in plants raised in soil inoculated with AM fungi (Table 3), with seedlings raised in the presence of *Gigaspora margarita* showing the most increase of all phytochemical constituents in the plant tissues. Such a variation in the phytochemical constituents in relation to the fungal species for other medicinal plant species is also well documented [1,27].

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Table 2. Influence of native AM fungi on P, K, Zn, Cu, and Fe content in shoot and root of *P. amboinicus*

Treatment	Phosphorus content (mg/plant)	Potassium content (mg/plant)	Zinc content (µg/plant)	Copper content (µg/plant)	Iron content (µg/plant)
Control (without AM fungi)	3.29[a]	3.2[a]	162.8[a]	61.7[a]	59.5[a]
Acaulospora bireticulata	5.50[b]	4.1[b]	195.0[bc]	74.9[b]	64.2[b]
Acaulospora scrobiculata	5.38[ab]	4.2[c]	198.5[b]	105.8[c]	72.8[c]
Gigaspora margarita	6.56[d]	5.4[d]	296.9[d]	112.3[d]	95.6[d]
Glomus aggregatum	6.23[d]	4.2[c]	280.9[c]	108.9[d]	92.2[d]
Glomus geosporum	5.95[c]	4.4[c]	241.3[bc]	98.5[c]	88.5[c]
Glomus mosseae	6.35[d]	4.6[c]	286.2[d]	109.2[d]	94.2[d]
Scutellospora heterogama	5.45[b]	3.4[a]	223.2[bc]	104.4[c]	88.2[c]

Note: Means (n = 5) in each column followed by the same letter are not significantly different ($p < 0.05$) from each other according to DMR test.

Table 3. Influence of different native AM fungi on phytochemical costituents in the leaves of *P. amboinicus*

Treatment	Total phenols (µg/g fresh wt.)	o-Dihydroxy-phenols (µg/g fresh wt.)	Flavonoids (µg/g dry wt)	Alkaloids (µg/g dry wt.)	Tannins (µg/g dry wt)	Saponins (µg/g dry wt.)
Control (without AM fungi)	95.0[a]	65.2[a]	3.12[a]	4.32[a]	0.280[a]	0.160[a]
Acaulospora bireticulata	120.5[c]	75.2[d]	3.25[b]	4.38[b]	0.290[b]	0.172[b]
Acaulospora scrobiculata	114.2[b]	70.2[b]	3.16[b]	4.36[b]	0.298[b]	0.176[b]
Gigaspora margarita	130.5[d]	85.4[d]	3.76[d]	5.12[d]	0.435[d]	0.210[d]
Glomus aggregatum	128.2[d]	82.3[d]	3.62[c]	4.86[c]	0.382[c]	0.195[c]
Glomus geosporum	118.5[b]	80.5[c]	3.58[c]	4.82[c]	0.365[c]	0.192[b]
Glomus mosseae	129.2[d]	83.4[d]	3.64[cd]	5.01[d]	0.395[d]	0.199[c]
Scutellospora heterogama	115.5[b]	72.72[b]	3.18[c]	4.38[b]	0.340[ab]	0.185[b]

Note: Means (n = 5) in each column followed by the same letter are not significantly different ($p < 0.05$) from each other according to DMR test.

Other items studied in relation to effects of soil inoculation with AM fungi are shown in Table 4. *Gigaspora margarita* and *Glomus mosseae* inhabited a significantly higher percentage of roots compared to other AM fungi. Similarly, spore number was also highest in the soil samples inoculated with *Gigaspora margarita* followed by soil inoculated with *Glomus mosseae*, indicating a better proliferating ability of these fungi with *P. amboinicus* as the host.

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Table 4. Effects of soil inoculation with AM fungi on per cent root colonisation, spore numbers in root zone soil, percent aggregation of rhizosphere soil, sturdiness quotient, biovolume and quality index of *P. amboinicus*

Treatment	AM fungi colonisation in root (%)	Number of AM fungi spores / 100 g of soil	Aggrega-tion of soil	Sturdiness quotient	Biovolume index	Quality index
Control (without AM fungi)	0^{a}	0^{a}	18^{a}	16.4^{a}	2442.2^{a}	0.45^{a}
Acaulospora bireticulata	53.5^{b}	320^{b}	36^{b}	16.8^{b}	2526.4^{b}	0.46^{b}
Acaulospora scrobiculata	45.5^{b}	310^{b}	39^{b}	16.9^{b}	2527.2^{b}	0.47^{b}
Gigaspora margarita	92.5^{d}	760^{d}	52^{d}	17.4^{d}	3245.4^{d}	0.58^{d}
Glomus aggregatum	85.5^{cd}	680^{cd}	48^{c}	17.1^{c}	3162.8^{c}	0.55^{c}
Glomus geosporum	82.0^{cd}	620^{cd}	42^{c}	16.9^{c}	2526.4^{b}	0.47^{b}
Glomus mosseae	88.5^{cd}	690^{cd}	45^{c}	17.2^{d}	2975.9^{b}	0.56^{c}
Scutellospora heterogama	63.0^{c}	460^{c}	32^{b}	16.6^{b}	2469.4^{b}	0.46^{b}

Note: Means (n = 5) in each column followed by the same letter are not significantly different ($p < 0.05$) from each other according to DMR test.

Mycorrhizal fungi are also implicated in soil structure improvement by increasing soil aggregation by their hyphae [28]. Soil aggregation is a measure of the amount of extramatrical hyphae, which is in turn related to the efficiency of the fungus [24]. This observation is further strengthened by the present study as mycorrhizal fungi used in this study significantly improved the aggregation of soil compared to that of the control (Table 4). Soil aggregation was highest in soil inoculated with *Gigaspora margarita* and *Glomus aggregatum* in that order. Other seedling parameters (sturdiness quotient, biovolume index and quality index) were also found to be all higher than those of the control, the increase being to the extent of 6.08%, 33.20% and 29.4% respectively (Table 4). Such values indicate a sturdier stem and a greater dry weight of the plant, qualities which are desirable among nursery seedlings [1,18].

AM fungi differ greatly in their symbiotic effectiveness which depends on their preference for particular soils or host plant specificity [29], direct ability to stimulate plant growth, rate of infection, competitive ability, and tolerance to applied chemicals. Giving weighting to quality index, but not neglecting the other parameters, *Gigaspora margarita* and *Glomus mosseae* were found to be the best and the next best fungus respectively for inoculating *P. amboinicus* in the nursery in order to obtain healthy, vigorously growing seedlings that could establish and perform better when planted in sandy loam soils.

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Conclusions

P. amboinicus seedlings show varied responses to different AM fungi, with *Gigaspora margarita* confering greater benefits compared to all other fungi used in this study. Further consideration of the ability for higher root colonisation, plant biomass, biovolume index, and mineral and phytochemical constituents suggested that a clear and specific relationship exists between a particular species of fungus and the plant.

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